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Neutrophilic Hypersegmentation Without Macrocytic Anemia

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In five months of examining some 25,000 blood smears of hospital and clinic patients, 200 patients were found whose blood had hypersegmented neutrophiles without macrocytosis of the erythrocytes. Sixty-five of these patients subsequently received adequate laboratory work-up. Seven proved to have a bacterial inhibitor in their serum which interfered with the microbiological assay of folic acid. Of these patients, six had normal B-12 levels. Of the remaining 58, 45 had a deficiency of folate, of vitamin B-12 or of both. In 13, no such deficiency could be established. Of these, seven proved to be uremic. It is concluded that the additional effort required to search carefully for hypersegmentation, even in the absence of erythroid macrocytosis, is thoroughly justified, and that a large percentage of those patients in whose blood hypersegmented neutrophiles are found will prove to have important deficiencies of folate or B-12, or will prove to be uremic.

HYPERSEGMENTATION OF THE NEUTROPHILES and macrocytic anemia traditionally go hand in hand in patients with chronic folate or vitamin B-12 deficiencies. Herbert,[1] during the course of producing experimental folate deficiency in a well-nourished volunteer, showed that the first hematologic abnormality to appear was neutrophilic hypersegmentation. This occurred long before the appearance of macrocytic anemia, presumably because of the greater life span of normal erythrocytes, as compared with the brief intra-vascular life of the neutrophiles. More recently,[2-4] hypersegmented neutrophiles have been noted in the blood of patients with the uremic syndrome, but without macrocytic anemia, and in some cases with only borderline deficiency of folate. In addition, patients receiving certain cytotoxic drugs may show megaloblastic changes in the face of normal folate and B-12 levels.

It would appear, therefore, that a diagnosis of early or acute folate and B-12 deficiency will be overlooked if macrocytosis and anemia are considered requirements for such a diagnosis. To detect such deficiencies, one should also look for hypersegmented neutrophiles in the blood of persons without macrocytic anemia.

Working on this thesis, and as part of our continuing emphasis on the importance of careful

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morphologic study,[5] we have searched for hypersegmented neutrophiles in all of the blood smears processed in our hematology laboratories. All smears thought by staff technicians to show signs of hypersegmentation have been examined by at least two additional trained observers. Particularly prominent in our series have been blood specimens showing absolute neutrophilic leukocytosis and toxic granulation without an increase in nonsegmented or "banded" neutrophiles. When we encountered hypersegmentation in the absence of macrocytosis, we sent to the clinical record a note describing our findings, and suggesting further study including serum folate and B-12 tests, and possibly a bone marrow examination.

In a period of about five months, during which technicians examined approximately 25,000 blood smears, we found 200 patients whose blood showed hypersegmentation without macrocytosis of the erythrocytes. This report summarizes our findings, and includes several characteristic case histories.

Methods*

All blood counts were done on the Coulter Counter Model S, which we have standardized against the cyanmethemoglobin technique, the microhematocrit, and a carefully standardized Coulter F^n for erythrocyte and leukocyte counts. We have stained all blood smears with Wright's stain, counter-stained with buffered Giemsa stain. We have excluded from our series all patients with a mean corpuscular volume (MCV) of 100 cubic microns (cu μ) or more. (Normal Mean $\pm$ 2. Standard deviation = 81.5 to 99.5 cu μ.)

We have arbitrarily set our definition of hypersegmentation at 4 percent or more of neutrophiles with five distinct lobes, connected by filaments, or of any cells with six or more lobes. We have determined the serum folate levels by microbiological assay against cultures of Lactobacillus casei, as described by Herbert.[6] Our normal range is 5 to 21 nanograms (ng) per ml of serum. We have determined serum vitamin B-12 levels by the radio-immunoassay technique of Tibbling,[7] our normal range being 180 to 800 picograms (pg) per ml.

*ADDENDUM: Since submitting this paper, we have changed our folate assay technique to the radio-assay method of Rothenberg et al [Rothenberg SP, DaCosta M, Rosenberg Z: A radio-assay for serum folate—Use of a two-phase sequential incubation, ligandbinding system. N Engl J Med 286:1335-1339, Jun 22, 1972]. In two months with this method, we have encountered ten additional cases of folate deficiency without macrocytosis. Their hemoglobins have ranged from 3.6 to 15.9 gms per 100 ml; their MCV's from 53 to 91 cu μ; their folate levels from 1.4 to 4.3 ng per ml (normal 4.5 to 12.9). All had normal B-12 levels.

TABLE 1.—*Findings in Patients with Hypersegmented Neutrophiles but Without Macrocytosis*

Diagnosis	*Number of Patients*
Positive Cases	45
Folate deficiency alone	29
Folate and iron deficiency	9
Folate and B-12 deficiency	4
Folate, B-12 and iron deficiency	1
B-12 deficiency alone	1
B-12 and iron deficiency	1
Negative Cases	13
Elevated BUN and Creatinine	7
BUN and Creatinine normal	6
Bacterial Inhibitor: No folate assay possible	7
B-12 normal	6
Not tested for B-12	1

Results

Of the 200 patients whose blood smears showed hypersegmented neutrophiles, 65 have subsequently received a reasonably thorough work-up, including tests of folate and B-12 levels and, in many cases, bone marrow examination. On the other hand, in 135 cases, our findings and suggestions were completely ignored. This happened because the patients were receiving cytotoxic drugs capable of producing hypersegmentation (16 cases); because the physician failed to see our note in a bulky clinical record or because the physician felt that the observation of hypersegmented neutrophiles in the absence of macrocytosis must surely be wrong.

Of the 65 patients who had a thorough work-up, seven proved to have a bacterial inhibitor in their serum which interfered with the microbiological assay of folic acid. Of these seven, six had B-12 assays, all of which showed normal levels. Of the remaining 58 patients, 45 proved to have a deficiency of folate, of vitamin B-12 or of both. In 13 patients, no such deficiency could be established. Of the 13, seven had elevated blood urea nitrogren and creatinine levels. Table 1 shows the distribution of findings among the 65 patients who were tested.

Reports of Cases

Case 1. Folate deficiency in acute alcoholic

A 55-year-old chronic alcoholic man was brought to the emergency room by members of the sheriff's department because of impending delirium tremens. On physical examination, the patient was essentially normal except for tremulousness. The spleen and liver were not palpably enlarged.

A blood count done shortly after arrival showed no anemia or macrocytosis. (Hemoglobin [Hgb]: 13.5 grams per 100 ml; MCV: 94 cu μ.) On the smear, however, we found many hypersegmented neutrophiles, and very few platelets. Because of these findings, we encouraged the patient's physician to order tests of serum folate and B-12 levels and a platelet count. The folate level (2.0 ng per ml) and platelet count (19,000 per cu mm) were both low, the B-12 level was normal.

A sternal bone marrow specimen proved to be hypoactive, with early megaloblastic changes in the erythroid cells, and with giant metamyelocytes and banded neutrophiles. Megakaryocytes were sparse, and those found did not appear to be producing platelets. There was distinct vacuolization of the proerythroblasts and myeloblasts. Stainable iron was increased, but we found no ring sideroblasts.

Admitted to hospital, the patient responded to paraldehyde, the hallucinations cleared and tremulousness decreased. Hemoglobin dropped over the next two days, however, as did platelet count, and the stools became guaiac positive. In addition, neutropenia developed. (Leukocytes: 2,000 per cu mm; neutrophiles: 46 percent.) The fibrinogen level was high normal, however, and tests for fibrin monomers and fibrin degradation products gave negative results, thus excluding disseminated intravascular coagulation. After six days of treatment with folic acid, and transfusion of two units of packed red cells, the hemoglobin had risen nearly to normal, the indices remained normal, and the leukocyte and platelet counts had also returned to normal levels. Hypersegmented neutrophiles had disappeared from the blood, and the stools were now guaiac negative. The patient was discharged on the tenth day on folate and multivitamins.

Comment

The demonstration of hypersegmented neutrophiles in a patient without macrocytosis or anemia called our attention to the fact that acute folate deficiency may occur without abnormality in the erythroid series. Such a picture must be common among alcoholics who have not been drinking long enough to develop the macrocytosis of alcoholic liver disease.

Case 2. Folate deficiency in alcoholic

A 58-year-old housewife was admitted to hospital for elective cholecystectomy and rectal polypectomy. For the past five months she had had persistent nausea, had vomited undigested food and had lost 50 pounds of body weight. She complained of inability to eat fatty foods, but stated that she liked and ate leafy vegetables. She admitted being a "binge drinker," however.

A blood count on admission showed normal values (Hgb: 14.2 grams; MCV 95 cu μ; mean corpuscular-hemoglobin concentration [MCHC]: 34.0 percent). There was a shift to the *right* in the neutrophiles, however, and we found appreciable numbers of hypersegmented neutrophiles.

The patient had cholecystectomy and rectal polypectomy without difficulty. On the sixth postoperative day, at a time when the patient was receiving neither vitamin supplement nor antibiotics, the B-12 level was normal (330 ng per ml) and the folate level was much decreased (less than 2 ng per ml). An aspirated bone marrow was normocellular, and showed early megaloblastic changes, most striking in the granulocytes. Marrow iron was normal in amount and distribution. With a diagnosis of inadequate folate intake, presumably a result of her drinking, the patient was discharged on one milligram of folic acid by mouth daily.

Comment

This patient's stores of folate very probably had been impaired by her drinking. The malnutrition associated with the loss of 50 pounds of body weight doubtless had contributed to the deficiency. Finally, the serum folate specimen was obtained after five days in the hospital during which time no solid food and or added vitamins were given. In any case, the finding of hypersegmented neutrophiles signaled an important deficiency.

Case 3. Folate deficiency and uremia

A 60-year-old black man, a retired sign-painter, came to the Medical Clinic because of persistent dizziness and headache. He had been under treatment for about a year for hypertension, receiving reserpine, methyl-dopa, guanethidine sulfate and potassium chloride. Recently, however, his hypertension had become refractory to this treatment. Further, he had developed fatigue on walking one block, and had lost twenty-five pounds of body weight in one year. The patient said that he did not use alcohol.

On examination, blood pressure was 240/120 in both arms. Mucosae were pale. The heart was regular at 85 beats per minute, and was somewhat enlarged on palpation and percussion. The lungs were clear, however, and no dependent edema

was noted. The liver had an overall height on percussion of 11 cm.

A blood count showed a hemoglobin of 6.9 grams; and MCV of 80 cu μ and an MCHC of 32.8 percent. There was modest neutrophilic leukocytosis, with slight left shift. (Leukocytes: 21,200 per cu mm; segmented neutrophiles: 69 percent; banded forms: 8 percent.) On the smear, however, we found a scattering of hypersegmented neutrophiles. Erythrocyte morphology was not particularly abnormal except for a lack of polychromasia. Our note to the patient's record stated that results of blood tests suggested azotemia, with folate deficiency superimposed.

Further laboratory studies showed blood urea nitrogen of 60 mg per 100 ml (normal: 10 to 20): a creatinine of 7.1 mg per 100 ml (normal: 0.7 to 1.4); and a low serum folate (less than 2.0 ng per ml). Normal results were obtained from tests of serum B-12 (800 pg per ml): serum iron and iron binding capacity (62 and 235 mgs per 100 ml; saturation: 26 percent); G-6-PD screen; L-E cell test; direct and indirect antiglobulin test and serum haptoglobin test. On hemoglobin electrophoresis, he proved to have sickle cell trait (Hgb A-S).

An aspirated bone marrow specimen showed normal cellularity with minimal megaloblastic changes, most evident in the granulocytic series. Stainable iron was present in normal amounts, but there were no sideroblasts.

We treated the folate deficiency with orally administered folate, but the azotemia and hypertension proved very resistant to therapy.

Comment

Some degree of hypersegmentation has been reported in many patients with uremic syndrome.[2-5] In this case the malnutrition which had caused a weight loss of 25 pounds doubtless contributed to a real folate deficiency.

Case 4. Iron and folate deficiency

A 21-year-old white school girl was admitted to hospital in acute status asthmaticus, one of many such admissions for this disorder. The patient reported having been given moderate doses of prednisone intermittently for several years, and in recent years had taken antacids because of duodenal ulceration and intermittent bleeding. Her diet was said to be adequate and her menses never excessively heavy. Results of recent stool guaiac tests had been negative. However, she had had persistent microcytic anemia for the past two years.

On this admission, she again showed a low grade microcytic, hypochromic anemia. (Hgb: 11.5 grams; MCV 74 cu μ; MCHC: 31.4 percent.) There was also a modest left shift in the neutrophiles, but there were significant numbers of five- and six-lobed neutrophiles on the smears.

The serum folate level proved to be low (less than 2.0 ng per ml), while the B-12 level was high (over 1,000 pg per ml). Tests of serum iron and total iron-binding capacity (TIBC) established the fact that there was definite iron deficiency. (Serum iron: 20 mg per 100 ml; TIBC: 310 mg per 100 ml; saturation: 6.5 percent.) An aspirated bone marrow showed generalized hyperplasia, with low-grade megaloblastosis, and with complete absence of stainable iron. Our diagnosis was (1) iron deficiency, probably due in part to heavy antacid therapy and (2) folate deficiency, presumably due to malabsorption related to chronic iron deficiency. She was discharged on oral iron and folic acid.

Comment

The apparently very significant folate deficiency was masked in this patient by the microcytosis related to iron deficiency. Such cases are probably by no means rare, as shown by our encountering nine in a period of five months.

Case 5. Folate deficiency in thalassemia minor

A 17-year-old unemployed black man came to the emergency room after being run over by a tractor. Bruises were noted on those parts of his body which the tractor wheel had passed over, and x-ray films of the skull showed a basal fracture. The patient said he did not use alcoholic beverages.

A routine blood count showed a microcytic, normochromic anemia. (Hgb: 11.8 grams per 100 ml; MCV: 67 cu μ; MCHC: 32.2 percent.) On the smears, there were many target and oval red cells, as well as scattered basophilic stippled cells. There also were moderate numbers of hypersegmented neutrophiles. Our note to the patient's record suggested that he be studied for a hemoglobinopathy, as well as for folate and B-12 deficiencies.

Hemoglobin electrophoresis showed type AA, with 3.6 percent Hgb A_2 (normal: 4 percent or less), and Hgb F 1.1 percent (normal: 1.0 percent or less). The serum folate level was definitely

low (2.0 ng per ml), while the B-12 and serum iron levels and iron binding capacity were normal. He refused a bone marrow examination.

Our hematologic diagnosis was (1) thalassemia minor, presumably of beta type and (2) coexistent folate deficiency, presumably due to inadequate dietary intake.

Comment

Just as the microcytosis of iron deficiency may mask any tendency toward macrocytosis, so may megaloblastosis remain hidden behind the microcytosis of thalassemia minor. It was the hypersegmented neutrophiles that led us to suspect folate deficiency.

Case 6. Folate deficiency in early pernicious anemia

A 76-year-old diabetic woman came to the drop-in clinic, complaining of weakness, shortness of breath and swollen ankles, all of one week's duration. She also reported dysuria, and that her urine at times had been bloody. She had been testing her urine for sugar, and what had usually been a 1 plus or 2 plus reaction had recently become 4 plus.

On physical examination, the only abnormal findings were that the liver was palpable 1 centimeter below the costal margin and that there was slight pitting edema of the ankles and feet. Vibratory sense was diminished in both feet, but results of the neurological examination were otherwise normal.

Results of complete blood count were completely normal, but there were significant numbers of hypersegmented neutrophiles on the smears. The fasting blood sugar was 335 mg per 100 ml.

Steps were taken to control the diabetes, and the requested laboratory work was ordered. Surprisingly enough, levels of both serum folate (4 ng per ml) and B-12 (70 pg per ml) were abnormally low. A Schilling test showed a 24-hour excretion of only 1 percent of the orally-administered radioactive vitamin B-12. Results of a second stage Schilling test were normal.

Our diagnosis was early pernicious anemia with some degree of malabsorption of folate, possibly due to the B-12 deficiency.

Comment

Whether the slight decrease in vibratory sensation would have led the clinician to a diagnosis of pernicious anemia is perhaps problematical. The discovery of hypersegmented neutrophiles certainly strengthened any suspicions.

Case 7. Iron and B-12 deficiency

A 75-year-old white man was admitted to hospital because of intermittent right arm weakness and expressive aphasia for the past three weeks. He also reported that there had been bright red blood in his stools for a matter of several months. On physical examination he appeared chronically ill and emaciated. Positive findings were otherwise confined to the neurological examination. There was decreased strength of the right arm, without tremors or fasciculations, and there was hyperreflexia on the right, with a positive Babinski. The rectal examination showed a stool positive for occult blood.

A complete blood count on admission showed a severe microcytic hypochromic anemia. (Hgb: 5.9 grams per 100 ml; MCV; 64 cu μ; MCHC: 27.5 percent.) The leukocyte and differential counts were normal, but again we found scattered hypersegmented neutrophiles. The serum iron studies confirmed the diagnosis of iron deficiency. (Serum iron: 23 mg per 100 ml; TIBC: 290 mg per 100 ml; saturation: 8 percent). The serum B-12, on the other hand, was also definitely low (110 pg per ml), with a normal folate (23.0 ng per ml). A bone marrow examination showed definite megaloblastosis, and a complete lack of stainable iron. The Schilling test showed no excretion of radioactive B-12 in 24 hours.

A barium enema examination showed a carcinoma of the colon at the hepatic flexure, while a brain scan showed an area of decreased perfusion in the left hemisphere, interpreted as indicating obstruction of the left middle cerebral artery.

The patient had hemicolectomy with transverse colostomy. No metastases were found on operation, and the post-operative recovery was satisfactory. We treated the blood picture with intramuscularly administered B-12 and oral iron, and the patient responded well. Results of a subsequent second stage Schilling test were normal at 15 percent excretion.

Comment

The severe iron deficiency in this old man, due to carcinoma, thoroughly masked the fact that he also had pernicious anemia. If the hypersegmented neutrophiles had not been found, the vitamin B-12 deficiency would probably have gone unrecognized for some time.

Case 8. Deficiency of iron, folate and B-12

This 52-year-old white woman was brought to the emergency room by her husband who had found her lying in a pool of blood on the kitchen floor. She had an eight-year history of a seizure disorder, for which she was receiving primidone. She and her husband both stoutly denied alcoholic intake. She complained of chronic diarrhea, worsened by eating green vegetables. She had lost weight from 115 to 93 pounds in the past two years.

On physical examination, laceration of the scalp was noted, but there was no evidence of fracture. Neurological examination showed hypertonic deep tendon reflexes in all extremities, and positive Babinski's bilaterally. Vibratory sense was lacking in both legs to the knees, and was sub-standard at the hips.

An initial blood count showed a normocytic, normochromic anemia. (Hgb: 7.3 grams per 100 ml; MCV: 84 cu μ; MCHC: 32.2 percent.) There was a shift to the right in the neutrophiles (banded cells: 0 percent), and many large, hypersegmented cells were found on the smears. We therefore determined the folate (2.0 ng per ml) and B-12 (80 pg per ml) levels, both proving abnormally low. Her serum iron and saturation were likewise low. (Serum iron: 23 mg per 100 ml; TIBC: 405 mg per 100 ml; saturation: 5.5 percent.) A bone marrow specimen obtained by sternal puncture, was frankly megaloblastic, and contained no stainable iron.

Our diagnosis, then, was a triple deficiency of folate, vitamin B-12 and iron, presumably due to some as yet undiagnosed malabsorption syndrome. A Schilling test showed 4.6 percent excretion of orally administered radioactive B-12 in 24 hours (normal: 7 percent or more). The patient, however, refused to undergo the second stage Schilling test, or any further testing of the gastro-intestinal tract, but insisted upon returning to the care of her family physician. The latter, on our advice, agreed to see that she received regular injections of vitamin B-12, folate and iron.

Comment

In this unusual case, it was the finding of hypersegmented neutrophiles which led directly to the further work-up, and to the discovery of the triple deficiency. The extent to which primidone might be contributing to the diarrhea, the neurologic disorder or even the megaloblastosis has not been determined.

Discussion

We undertook this study anticipating that we would discover an occasional case of folate deficiency, largely among alcoholics. We were not particularly surprised at the failure of many of our colleagues to follow our suggestions regarding further laboratory investigation. The fact of folate or B-12 deficiency without macrocytic anemia has indeed received very little attention.

We were surprised to find, among the 58 patients who were adequately studied, and who did not have a bacterial inhibitor which interfered with the folate assay, that 43 had a definite folate deficiency. Even more surprising were the seven who proved to have a low vitamin B-12 level. This experience indicates to us that the additional effort required to look carefully for hypersegmentation is thoroughly justified in the additional help the laboratory can give the clinician.

We were particularly interested in the 13 patients in whose blood we found hypersegmentation, but whose serum folate and B-12 levels proved normal. Some may have had blood specimens for folate determination drawn sufficiently long after the start of a nutritious hospital diet that a formerly low folate level had come into the normal range. Seven of the thirteen, however, proved to be uremic to at least some extent, suggesting that uremia must be included in the differential diagnosis when hypersegmentation is encountered in the absence of macrocytosis.

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